THE RELATIONSHIP BETWEEN ADIPONECTIN TO LEPTIN RATIO, METABOLIC DYSFUNCTION, AND DIET IN THE PEDIATRIC OBESE

by

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STATEMENT OF THESIS APPROVAL

The following faculty members served as the supervisory committee chair and members for the thesis of Brittney Marie Urban.

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ABSTRACT

Adipokines, including adiponectin and leptin, are secreted by adipose tissue. With an increase in fat mass, concentrations of adiponectin decrease and leptin increase, altering the adiponectin/leptin (A/L) ratio. A/L ratio is associated with metabolic dysfunction, as are multiple diet factors including breastfeeding in infancy and breakfast intake. However, it is not known whether these diet factors are related to A/L ratio, and if they will protect against metabolic dysfunction in a population at an increased risk. The purpose of this study was to investigate whether breastfeeding duration in infancy and current breakfast frequency are associated with A/L ratio and metabolic dysfunction in children and adolescents with obesity.

The study cohort consisted of obese (body mass index ≥ 95th percentile) children and adolescents (n = 100, 46M). Fasting blood samples were taken to measure high density lipoprotein cholesterol, low density lipoprotein cholesterol, triglycerides, insulin, glucose, adiponectin, and leptin levels. Weight, height, and blood pressure were also assessed. Breastfeeding history was obtained through the National Health and Nutrition Examination Survey of 2003-2004, and breakfast frequency was reported through the Gold Medal School Parent Questionnaire administered to parents.

Of the 100 participants, 82% were breastfed as infants. Among the breastfed, 66% (n = 54) were breastfed for ≥ 6 months and 62% (n = 54) were breastfed exclusively for ≥ 6 months (4 parents could not recall duration of exclusive breastfeeding). Of the

breakfast responses collected (n = 46), most participants (59%, n = 27) reported consuming breakfast every day. There was no association between these diet factors and A/L ratio or the presence of metabolic dysfunction. Differences in A/L ($p = 0.02$) and leptin ($p = 0.009$) were noted between categorical breakfast frequencies when gender was controlled for, and there was a significant difference in BMI between categorical breakfast intakes ($p = 0.02$). There were no significant relationships in the linear model of breakfast. No association between A/L ratio and metabolic dysfunction was observed. In conclusion, breastfeeding duration and breakfast frequency do not appear to influence A/L ratio or metabolic dysfunction in this population of obese children and adolescents.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

INTRODUCTION

The prevalence of obese children and adolescents (age 2-19) in the United States has almost tripled since 1980, with nearly 17% of this age group obese as of 2009 (1). Obesity, particularly central obesity, is one risk factor for metabolic syndrome in adults (2) and children (3). Metabolic syndrome is characterized by a cluster of risk factors that, when occurring simultaneously, increase the risk of chronic diseases such as type II diabetes, coronary artery disease, and stroke. In adults, the syndrome is well defined (2); however, consensus is lacking over the appropriate manner to define and diagnose metabolic syndrome in youth (4).

In children, markers of metabolic dysfunction, especially those relating to cardiovascular disease (CVD) and insulin resistance, may be used to determine risk of the above chronic diseases in lieu of a metabolic syndrome diagnosis (4). Markers of metabolic dysfunction include dyslipidemia (decreased high density lipoprotein cholesterol [HDL], elevated low density lipoprotein cholesterol [LDL], and elevated triglycerides), elevated blood pressure, insulin resistance (4), and elevated waist to height ratio (WHR) (5). A number of factors have been associated with an altered risk of metabolic dysfunction. LDL cholesterol has been observed to decrease with a greater consumption of monounsaturated (6) and polyunsaturated fatty acids (7), as well as with a greater intake of nuts (8); the Dietary Approaches to Stop Hypertension (DASH) diet, along with a daily 500 calorie restriction, may increase HDL and decrease triglycerides

and blood pressure (9); a high intake of fiber may lower insulin and postprandial glucose levels (10, 11); and daily consumption of sugar sweetened beverages has been associated with an increased risk of metabolic syndrome, elevated blood pressure, high triglycerides, and low HDL (12). Serum adiponectin, leptin, and diet factors such as breastfeeding in infancy and breakfast intake are also known to influence metabolic dysfunction risk.

Adiponectin and leptin are two adipokines that have been linked to metabolic health. These adipokines are produced and secreted by adipose tissue (13); however, as the amount of white adipose tissue increases, serum adiponectin concentration decreases (14) while leptin increases (15). In adolescents, an inverse relationship has been observed between adiponectin and triglycerides, systolic blood pressure, and WHR, along with a positive relationship to HDL levels, regardless of body mass index (BMI) (16). In adults, high adiponectin levels appear to protect against insulin resistance and heart disease (17, 18), while low levels are associated with dyslipidemia, elevated blood pressure, type II diabetes, and coronary artery disease (17, 19).

Blood concentrations of leptin and adiponectin can also be measured as a ratio to predict risk of metabolic dysfunction using the A/L ratio. Low adiponectin and high leptin levels (a low A/L ratio) may be a predictor of atherosclerosis in healthy adult males (20) and has been related to a greater risk of CVD and the associated risk factors in both adults and children (21, 22, 23). A low A/L ratio has been correlated with the presence of metabolic syndrome (24, 25, 26, 27), and has been observed to be a better predictor of insulin resistance than HOMA-IR or adiponectin and leptin levels alone (28, 29).

Infant nutrition, particularly breastfeeding, may also influence risk of metabolic disease later in life. Research suggests that the risk of chronic disease, such as type II

diabetes and CVD, is lower if an individual was breastfed during infancy (4, 30, 31, 32, 33). A history of breastfeeding may promote a lower cholesterol and BMI in adulthood (4), and the World Health Organization concluded that a longer duration is associated with lower total cholesterol, blood pressure, and prevalence of overweight or obesity (33).

Additionally, the consumption of a daily breakfast is a dietary pattern that can impact risk of disease and metabolic dysfunction in adolescence. Evidence supports an inverse relationship between frequent breakfast intake and overweight/obesity (34, 35, 36). A history of skipping breakfast throughout life has been linked to markers of metabolic dysfunction that include elevated waist circumference, fasting insulin, total cholesterol, LDL, and decreased HDL (37, 38). In children and adolescents with obesity, skipping meals (primarily breakfast) may also be associated with higher fasting glucose and triglycerides (39).

A history of breastfeeding and regular breakfast consumption appear to be beneficial in minimizing metabolic dysfunction, but additional research is necessary. For example, breastfeeding has been negatively associated with obesity in some studies (40, 41, 42), while in others the results were slight or not statistically significant (43, 44). Contradicting results were also observed between breastfeeding and HDL (40, 45), LDL, triglycerides, and blood pressure (45). Similarly, while breakfast has been associated with a healthy weight (34, 35, 36), a large cross sectional analysis of breakfast consumption in Korean adolescents found no correlation between frequency of breakfast intake and body weight (46). Finally, little research has been conducted on the benefits of a history of

breastfeeding and breakfast intake in those at increased risk of metabolic dysfunction, namely, children who are already obese.

Although A/L ratio and diet have individually been found to modify risk of metabolic dysfunction, it is not known if diet and A/L ratio are associated. Research supports an association between diet and adiponectin levels, including a positive association with greater consumption of fiber and low glycemic index foods (47), whole grains (48), a higher score on the Healthy Eating Index (49), and adherence to the Mediterranean diet (48, 50). Negative associations were noted with greater refined cereal consumption (51). Investigations into dietary habits like breastfeeding and breakfast consumption, however, are limited. Most literature on breastfeeding is focused on adiponectin concentrations between different breastfeeding durations in infants. In addition, there has been only one study suggesting that consuming an early meal (such as breakfast) may beneficially influence serum adiponectin in obese individuals for at least 3 hours (52).

Further research is needed to clarify the relationship between diet, A/L ratio and metabolic dysfunction, and to determine if diet and A/L ratio are linked. Specifically, little is known about the impact infant breastfeeding and breakfast consumption may have on A/L ratio in the pediatric obese. The aim of this study was to investigate the relationship between dietary factors (including breastfeeding duration and breakfast frequency) and A/L ratio in a cohort of obese children and adolescents. In addition, this study explored the degree that these dietary factors and A/L ratio influence metabolic dysfunction in obese youth.

METHODS

Research Design

This cross sectional study examined the relationship between diet, the adiponectin/leptin (A/L) ratio, and metabolic dysfunction during puberty in obese (BMI $\geq 95^{th}$ percentile for age and gender) children and adolescents age 8-17 years. The specific dietary measures collected include a history of breastfeeding duration in infancy and current average frequency of breakfast intake. Diet was evaluated through the use of questions from validated questionnaires. Serum lipids, insulin, glucose, adiponectin and leptin were collected via fasted blood draw. Degree of metabolic dysfunction was determined using serum lipids (HDL, LDL, and triglycerides), blood pressure, insulin resistance (HOMA-IR), and WHR. Participants were enrolled between the period of July 15, 2010 and January 31, 2013.

Participants were recruited from Salt Lake County in the following venues: as family members of a research cohort of extremely obese adults (followed by Dr. Steve Hunt, Cardiovascular Genetics Center, University of Utah), the University of Utah Adolescent Preventive Cardiology Clinic (APCC), the University Pediatrics Clinic, community pediatric healthcare providers, public buildings, wellness facilities, the Catholic Diocese, and universities and colleges in Salt Lake County. Enrollment in the study occurred following informed parental consent and child assent. After informed

consent was completed, baseline data was collected. This study protocol was approved by the University of Utah Institutional Review Board for Human Subjects (IRB#31720).

Subject Selection Criteria

Obese (BMI ≥ 95th percentile for age and gender) children and adolescents (n=100), both male (n=46) and female (n=54), between the ages of 8 and 17 at the time of recruitment, were eligible for this study. Participants were required to be in good health other than insulin resistance, dyslipidemia, impaired glucose tolerance, or hypertension. Exclusion criteria included refusal to participate, an inability to speak either English or Spanish, a genetic syndrome or other endocrine disorder known to cause obesity (Cushing's, Prader Willi, leptin deficiency, etc.), hypothyroidism, cystic fibrosis, history of pregnancy, cancer or history of cancer, active infectious disease, a history of cardiovascular disease or stroke during the previous 36 months, triglycerides > 400 mg/dL, diabetes mellitus (types I and II), and the use of psychotropics, sulphonylureas, thiazolidinediones, insulin, glucocorticoids, antineoplastic agents, angiotensin receptor blocker, or angiotensin converting enzyme inhibitors.

Screening Process

Patients were identified from the APCC and the University Pediatrics Clinic by chart review or by a pediatrician. All potential participants were assessed for inclusion and exclusion criteria over the phone using a standardized instrument. Participants who qualified were then invited to schedule an appointment at the Center for Clinical and Translational Science (CCTS), Oquirrh View Community Health Center, or St. Joseph the Worker Catholic Church for data collection.

Data Collection

Anthropometrics

Height was measured to the nearest 0.1 cm with a stadiometer (Model Height-Rite 225, Seca, Culver City, CA). Waist circumference was obtained by wrapping a measuring tape around the torso at the level of the umbilicus. The measurement was recorded at the end of expiration while the tape was held snugly without compressing the skin. Height and waist circumference were each taken twice; if the difference between the two measurements was greater than 1 cm, a third measurement was performed and an average of measurements was used. Waist circumference and height were used to calculate the WHR. Weight was obtained using a digital scale (Model 5002, Scale-Tronix, White Plains, NY). BMI was calculated as kg/m^2. Blood pressure was measured two times in one arm using a sphygmomanometer (Model Dynamap Pro 400, GE, Fairfield, CT). If the difference between the measurements was greater than 5 mmHg, a third measurement was performed and an average of the measurements was used. All measurements were obtained by a trained research assistant.

Biomarkers

Blood draws were obtained from participants following an overnight fast of at least 12 hours via standard CCTS procedures. Specifically, an intravenous catheter was placed in the arm to collect blood for measurement of fasting serum concentrations of insulin, glucose, adiponectin, leptin, and a complete lipid profile.

Insulin, adiponectin, and leptin concentrations in sera were measured in an enzyme linked immunosorbent assay format by Dr. Cynthia Chappell at the University of Texas at Houston using commercially available kits (Alpco Diagnostics, Salem,

NH). These kits have intraassay precision of 1.0 – 7.4% and interassay precision of 2.4 – 8.4%. The sensitivity of the assays is 1.5 ng/mL; accuracy is 92 – 100%. Procedures for each assay were carried out according to the manufacturer's instructions. Each serum sample was assayed twice. All concentrations were derived from a standard curve run on the same plate. Samples exceeding the highest concentration of the standard curve were diluted 1:2 and reassayed in duplicate. Standard curves for all plates yielded an R^2 value of ≥0.98. The coefficient of variation for TC and triglycerides are <2%, and <5% for HDL (53). LDL was calculated using the Friedwald equation. Lipid analysis was performed in the Cardiovascular Genetics Research Center Laboratory using a manual method (colorimetric for TC and triglycerides, dextran sulfate precipitation method for HDL). Glucose was measured using a glucose analyzer. Serum adiponectin and leptin were used to calculate the A/L ratio. Insulin and glucose were used to calculate HOMA-IR (an estimate of insulin resistance).

Metabolic Dysfunction

Metabolic dysfunction was defined as the presence of at least three abnormal measurements of lipids, blood pressure, insulin sensitivity, or WHR. Specific cutoffs for measurements included, HDL ≤ 40 mg/dL, LDL ≥ 110 mg/dL, triglycerides ≥ 75 mg/dL for children 9 years of age and younger and ≥ 90 mg/dL for children age 10 and up, blood pressure ≥ 90^{th} percentile or > 120/80 mmHg (4), HOMA-IR ≥ 3.16 (54), and a WHR ratio ≥ 0.5 (55, 56).

Breastfeeding History and Dietary Intake

Nutrition history was obtained after the blood draw. The participant or the parent/guardian completed a paper copy of the nutrition questionnaire while a research assistant was present to answer questions. Breastfeeding history was reported via questions from the National Health and Nutrition Examination Survey Diet Behavior and Nutrition Survey of 2003-2004 (57). Parents reported breastfeeding history through survey questions regarding the child's age at first food or beverage other than breast milk (exclusive breastfeeding duration) and age at complete cessation of breastfeeding (total breastfeeding duration). If breastfeeding history questions were missed during the scheduled appointment, the parent/guardian was contacted by a trained research assistant by phone or email to collect the missing data. The average days per week the participant consumes breakfast was obtained using questions from the Gold Medal School Parent Questionnaire (Appendix) (58).

Statistical Analysis

Means and standard deviations of lipids, blood pressure, insulin, glucose, adiponectin, leptin, A/L ratio, history of breastfeeding duration, and breakfast frequency were computed. A generalized linear model was used to model BMI, adiponectin, leptin, and A/L ratio in terms of breastfeeding duration (both total and exclusive) and breakfast frequency while also controlling for ethnicity and gender. Fisher's F-test was applied to check if the presence and duration of breastfeeding and breakfast intake were significantly related to BMI, adiponectin, leptin, and A/L ratio. Breakfast frequency was also treated as categorical data and analysis of variance was applied.

Logistic regression was applied to predict the presence of metabolic dysfunction (with ≥ 3 and ≥ 4 abnormal measures) using breastfeeding, breakfast intake, and A/L ratio as predictors. Chi-square tests were applied to test if these predictors had a significant relationship with metabolic dysfunction. Statistical analyses were performed using SAS statistical software (version 9.3, Cary, NC). Statistical significance was defined as $p < 0.05$.

RESULTS

A total of 100 children and adolescents participated in the research study. Table 1 details participant demographics. Ages ranged from 8 – 17 years, with 61% aged 8 – 12 and 39% aged 13 – 17. Every participant had BMI $\geq 95^{th}$ percentile, with absolute values ranging from 10.2 – 47.7 kg/m^2. Females comprised over half of the sample (54%, n = 54). All races and ethnicities were included; however, the cohort was predominantly Hispanic (54%, n = 54) and non-Hispanic White (33%, n = 33).

Table 2 depicts the average scores of metabolic dysfunction markers and A/L ratio in the cohort. With respect to the cutoffs for metabolic dysfunction markers in youth, the average measurement of triglycerides (126.4 ± 69.5 mg/dL), HOMA-IR (4.7 ± 3.5), and waist to hip ratio (0.63 ± 0.08) in this cohort exceeded the cutoffs for the normal range of measurements (see methods). Means of HDL, LDL, and blood pressure

Table 1
Participant Characteristics

Age	**Years**
Mean ± SD*	11.7 ± 2.7
BMI	**kg/m^2**
Mean ± SD*	28.8 ± 6.1
Gender	**(%)**
Male	46.0 (n = 46)
Female	54.0 (n = 54)
Ethnicity	**(%)**
Hispanic	54.0 (n = 54)
White, Non-Hispanic	33.0 (n = 33)
Other/No Response	13.0 (n = 13)

*SD = Standard Deviation

Table 2
Average Values of Metabolic Dysfunction Markers

Metabolic Marker	Mean ± SD*
HDL (mg/dL)	46.4 ± 10.6
LDL (mg/dL)	94.2 ± 20.7
Triglycerides (mg/dL)	126.4 ± 69.5
Systolic Blood Pressure (mmHg)	111.6 ± 11.5
Diastolic Blood Pressure (mmHg)	66.8 ± 8.2
Systolic Blood Pressure Percentile (%)	58.5 ± 27.5
Diastolic Blood Pressure Percentile (%)	59.6 ± 24.2
HOMA-IR	4.7 ± 3.5
WHR	0.63 ± 0.08
A/L Ratio	0.4 ± 0.7

*SD = Standard Deviation

were within the suggested range for children and adolescents. The mean A/L ratio was 0.4 ± 0.7.

Parents, or legal guardians, of all 100 participants completed breastfeeding questions during the baseline assessment. Of these 100 subjects, 82 (82%) were breastfed as infants. Parents of study participants also reported the total length of time the child was breastfed (from 1 week to ≥ 12 months), along with the length of time spent exclusively breastfeeding (from 1 week to ≥ 6 months). The average total time spent breastfeeding in infancy for these individuals was 8.3 months; the average time spent exclusively breastfeeding was 3.7 months. Table 3 outlines participant responses to the NHANES breastfeeding duration questions. A breastfeeding duration of 6 months or greater was seen in 66% (n = 54) of participants who were breastfed. Exclusive breastfeeding for at least 3 months was reported in 62% (n = 51) of participants, but fell to 34% (n = 28) at 6 months or greater. There were no statistically significant differences in BMI among breastfeeding groups.

Table 3
Breastfeeding Duration and Corresponding A/L Ratio

Breastfeeding Duration	N	Adiponectin (μg/mL)	Leptin (ng/mL)	A/L Ratio
Not Breastfed	18	6.6 ± 2.7	37.7 ± 25.9	0.31 ± 0.28
Total Breastfeeding	82			
< 3 Months	14 (17%)	7.2 ± 2.6	43.1 ± 33.3	0.33 ± 0.37
3 to < 6 Months	14 (17%)	5.4 ± 2.6	26.7 ± 22.6	0.63 ± 1.14
≥ 6 Months	54 (66%)	7.1 ± 3.7	46.9 ± 41.7	0.40 ± 0.66
Exclusive Breastfeeding	78			
< 3 Months	27 (35%)	6.9 ± 2.5	49.8 ± 41.3	0.32 ± 0.44
3 to < 6 Months	23 (29%)	6.3 ± 3.5	47.9 ± 47.8	0.52 ± 0.96
≥ 6 Months	28 (36%)	7.2 ± 4.2	31.8 ± 21.4	0.47 ± 0.74

Table 3 also summarizes the average corresponding adiponectin, leptin, and A/L ratio of different durations of total and exclusive breastfeeding times in the participants (4 parents could not recall duration of exclusive breastfeeding). In the analysis, breastfeeding duration was split into 7 groups; "not breastfed" and 3 groups within total and exclusive breastfeeding time subcategories that included: > 0 to < 3 months, ≥ 3 months to < 6 months, and ≥ 6 months. The average A/L ratio was 0.31 for those who were never breastfed and 0.43 for those who were breastfed for any amount of time; however, this difference was not statistically significant. When the means of adiponectin, leptin and A/L ratio were compared using Fisher's F-test, there was no difference due to total breastfeeding time or exclusive breastfeeding duration in this population. There were no statistically significant differences due to gender or ethnicity in breastfeeding duration, adiponectin, leptin, or A/L ratio.

Breastfeeding duration was also compared to metabolic dysfunction. A total of 86 participants had complete metabolic measurements, and were thus included in the

analysis. Metabolic dysfunction was defined as ≥ 3 abnormal measures of HDL, LDL, triglycerides, blood pressure, HOMA-IR, and WHR. Presence of metabolic dysfunction (with ≥ 3 abnormal measures) was not associated with breastfeeding, total breastfeeding duration, or exclusive breastfeeding duration using Chi-square tests. There was also no association between breastfeeding, total breastfeeding duration, or exclusive breastfeeding duration and the presence of ≥ 4 abnormal metabolic measures. A summary of metabolic dysfunction presence can be found in Table 4, with both the number of participants in each category, along with the proportion of each group that qualified as having metabolic dysfunction.

Regarding breakfast consumption, a total of 46 breakfast frequency responses were obtained during data collection from study participants (n =100). Table 5 summarizes breakfast consumption responses and the average corresponding A/L ratio. Of the 46 subjects who answered the question on breakfast intake, only 9% (n = 4) stated

Table 4
Breastfeeding and Presence of Metabolic Dysfunction*

Breastfeeding Duration (number of participants)	≥ 3 Abnormal Values	≥ 4 Abnormal Values
Not Breastfed (n = 18)	33%	17%
Total Breastfeeding (n = 82)		
< 3 Months (n = 14)	36%	14%
3 to < 6 Months (n = 14)	36%	7%
≥ 6 Months (n = 54)	39%	11%
Exclusive Breastfeeding (n = 78)		
< 3 Months (n = 27)	39%	13%
3 to < 6 Months (n = 23)	30%	4%
≥ 6 Months (n = 28)	43%	14%

No differences between breastfeeding groups
*Metabolic dysfunction includes HDL, LDL, triglycerides, blood pressure, HOMA-IR, WHR

Table 5
Breakfast Consumption and A/L Ratio

Breakfast Frequency, days/week	N	Adiponectin (μg/mL)	Leptin (ng/mL)	A/L Ratio
0 Days	4	5.3 ± 2.2	53.3 ± 52.3	0.18 ± 0.19
1 Day	1	2.0	14.9	0.13
2 Days	0	-	-	-
3 Days	1	7.8	16.7	0.47
4 Days	3	9.5 ± 2.7	15.6 ± 3.4	0.65 ± 0.34
5 Days	5	7.9 ± 1.8	24.5 ± 13.3	1.08 ± 1.79
6 Days	5	8.4 ± 5.1	15.6 ± 11.1	0.74 ± 0.71
7 Days	27	6.4 ± 2.5	34.1 ± 32.4	0.32 ± 0.28

that they do not consume breakfast. The majority of participants (59%, n = 27) reported consuming breakfast every day. Average breakfast consumption was 5.6 days per week for these 46 individuals. Corresponding adiponectin, leptin, and A/L ratio were obtained for 45 of those who reported breakfast consumption.

The groups with the lowest A/L ratio were those who reported consuming breakfast 1 day a week (A/L of 0.13) or no breakfast at all (A/L of 0.18 ± 0.19); the highest average A/L ratios were seen in the groups consuming breakfast 5 or 6 times weekly (1.08 ± 1.79 and 0.74 ± 0.71, respectively), although there was a large standard deviation and these differences were not statistically significant. Analyses of breakfast consumption and A/L ratio were conducted using a generalized linear model with breakfast as a categorical predictor and as a continuous predictor. When F-tests were applied to test for differences in A/L ratio and leptin concentration between different breakfast frequencies, treating days of breakfast consumption as a categorical variable and after controlling for gender, there was a significant difference ($p = 0.02$ and $p = 0.009$, respectively). In addition, there was a significant difference in BMI between

different breakfast intakes ($p = 0.02$); those consuming breakfast 3 – 5 days per week had a lower BMI (24.4 kg/m^2) than those who consume less breakfast (0 – 1 times per week, 32.7 kg/m^2) and the most breakfast (6 – 7 times per week, 27.2 kg/m^2). However, these differences did not persist when breakfast consumption was treated as a linear predictor. With breakfast as a linear predictor, there was a relationship between breakfast frequency and A/L ratio, when controlling for ethnicity ($p = 0.05$). There were no significant differences in adiponectin, leptin, or A/L ratio with increasing amounts of breakfast consumption in the study population (Table 5).

Metabolic dysfunction was also measured in relation to breakfast intake (Table 6). Of the 46 breakfast responders, 33 had complete metabolic dysfunction measurements. Chi-square tests revealed no association between the presence of metabolic dysfunction (with ≥ 3 abnormal measures) and the amount of weekly breakfast consumption. There was also no association between breakfast intake and the presence of ≥ 4 abnormal metabolic measures. A summary of metabolic dysfunction can be found in Table 6, with

Table 6
Breakfast Consumption and the Presence of Metabolic Dysfunction*

Breakfast Frequency, days/week (number of participants)	≥ 3 Abnormal Values	≥ 4 Abnormal Values
0 Days (n = 4)	25%	25%
1 Day (n = 1)	0	0
2 Days (n = 0)	-	-
3 Days (n = 1)	100%	100%
4 Days (n = 3)	33%	0
5 Days (n = 5)	40%	0
6 Days (n = 5)	20%	0
7 Days (n = 27)	37%	11%

*Metabolic dysfunction includes HDL, LDL, triglycerides, blood pressure, HOMA-IR, WHR

both the number of participants in each category, along with the proportion of each breakfast group that qualified as having metabolic dysfunction.

Finally, logistic regression was applied to predict the presence of metabolic dysfunction using A/L ratio as a predictor. Using Chi-square tests, there was no significant relationship between A/L ratio and metabolic dysfunction as defined as ≥ 3 abnormal values, or as ≥ 4 abnormal values. The effects due to gender and ethnicity were unclear through the logistic regression analysis, due to a sample size that was too small for a stable result.

DISCUSSION

This study investigated the effect of diet, specifically breastfeeding and breakfast, on A/L ratio and metabolic dysfunction in obese youth. Additional analyses between A/L ratio and the presence of metabolic dysfunction were also performed. Statistical tests revealed no association between the length of total or exclusive breastfeeding in infancy and alterations in A/L ratio or the presence of metabolic dysfunction in childhood and adolescence. Similarly, a greater frequency of breakfast intake was not predictive of alterations in A/L ratio or metabolic dysfunction. A/L ratio was not associated with the presence of metabolic dysfunction as well.

There were no statistically significant differences due to gender or ethnicity in the relationship between breastfeeding duration, adiponectin, leptin, and A/L ratio. In addition, there were no statistically significant differences in BMI among breastfeeding groups. There were however, differences due to gender and ethnicity in comparisons of breakfast intake, leptin, and A/L ratio, as well as differences in BMI among different breakfast frequencies. When breakfast intake was examined categorically, differences were observed in BMI across the various frequencies of breakfast consumption, with the lowest BMI in those consuming breakfast 3-5 times per week (24.4 kg/m^2) ($p = 0.02$). Differences were also observed in A/L ratio ($p = 0.02$) and leptin ($p = 0.009$) across the possible breakfast intakes when gender was controlled for. These categorical associations disappeared when breakfast frequency was treated as a quantitative variable in a general

linear model. In the analysis of breakfast intake as a linear model, there were no statistically significant associations. Of interest, however, is a relationship between breakfast frequency and A/L ratio when ethnicity was controlled for ($p = 0.05$).

The observed results do not support studies that suggest breastfeeding and breakfast consumption offer a benefit to metabolic health. Current research proposes that a history of being breastfed may promote a lower cholesterol, BMI (4, 33), and blood pressure (33). However, with no statistically significant relationship found between breastfeeding and metabolic dysfunction, the present study agrees instead with Parikh et al. and Rudnicka et al. who found no association between breastfeeding and HDL (40, 45), LDL, triglycerides, and blood pressure (45). Participants in current research of metabolic dysfunction and breastfeeding history tend to be adults, not a population of obese children and adolescents, which may explain the differences observed. Differences in the present study may also be explained by variations in weight of the sample population, as breastfeeding has previously been associated with a decreased prevalence of overweight and obesity (34, 35, 36).

Similar to breastfeeding, results detailing breakfast consumption in relation to metabolic dysfunction did not reflect findings of current literature. Skipping breakfast has been associated with components of metabolic dysfunction that include an increased LDL, fasting insulin, and waist circumference, as well as decreased HDL (37, 38). Specifically in children, skipping breakfast is associated with higher triglycerides and fasting glucose (39). The present study did not measure trends of breakfast frequency in relation to individual metabolic factors; instead, the outcome of interest was the presence of a minimum number of abnormal values to qualify as metabolic dysfunction. Although

results are inconsistent with the literature, a comparison of individual factors to a sum of factors is likely a contributing cause to the discrepancy. Another explanation for the results could include participant responses to the breakfast frequency question – most individuals reported regular consumption of breakfast all days of the week. With few respondents of other frequencies, comparisons of groups were limited. A lack of significant findings may also be explained by the narrow research question, as the type of breakfast consumed may impact metabolic dysfunction more than frequency alone. A study by Deshmukh-Taskar et al. found that while breakfast consumption in general provided benefits in cholesterol levels, the consumption of ready to eat cereals (rather than other types of breakfast) was associated with additional benefits in weight and blood pressure (38).

In addition to diet, research suggests a relationship between metabolic dysfunction and the A/L ratio as well as adiponectin levels. Higher adiponectin is associated with many metabolic benefits including improved HDL, triglycerides, systolic blood pressure, and WHR in adolescents (16); low A/L ratio is associated with metabolic syndrome (24, 25, 26, 27) and insulin resistance (25, 27, 28, 29). However, this was not seen in the study results. The present study found no relationship between A/L ratio and metabolic dysfunction in obese youth. The associations observed with metabolic dysfunction were witnessed in adult populations, not a cohort of obese youth. Furthermore, puberty is a time of hormonal change, and adiponectin and leptin levels have been noted to differ with age during this period, potentially skewing results (59).

Associations between A/L ratio and breastfeeding history or breakfast intake in literature are less clear. Although research suggests that adiponectin may be influenced

by diet, no studies have investigated A/L (or adiponectin) in relation to breastfeeding history or breakfast habits in children or adolescents. One study by English et al. found that the consumption of an early meal helped normalize serum adiponectin levels in obese adults for at least 3 hours (52). Nevertheless, conclusions were drawn from nonfasting measurements of adiponectin and the results reflected short term levels of the adipokine. Current literature may, however, offer an explanation for the association between A/L ratio and categorical levels of breakfast intake between genders. A study by Mirza et al. found significant differences in adiponectin and leptin between males and females, similar to results found in this study (24). The observations in this case would be due to varying levels of adipokines between genders.

Research is lacking in the comparison of diet, A/L ratio, and metabolic dysfunction. Fewer studies exist in this research area that examine a population of obese children and adolescents. Particular attention to this population is critical at this point in time, with the high rates of pediatric obesity and associated metabolic consequences in the United States. Additional explorations are needed to expand the knowledge base of metabolic dysfunction and diet in this group. Specifically, additional research on breakfast and metabolic health, expanding upon frequency to include type and amount of breakfast consumed, is recommended.

Strengths and Limitations

Strengths of the study include the use of validated nutrition questions and a targeted sample of obese children and adolescents. Other strengths include a unique research topic with a study cohort that has not been investigated extensively in literature. Although results were statistically insignificant, the current study on the effects of diet on

metabolic dysfunction in youth suggests a new area of research in the quest for greater understanding of obesity and metabolic disease in children. This study also focused on specific dietary factors (breastfeeding in infancy and breakfast consumption), rather than a generalized healthful diet, as a more applicable potential method in the treatment and prevention of childhood obesity.

The study was limited by the use of questionnaires, rather than researcher observation or diet records, to assess diet. The reporting of breastfeeding history was a limitation, requiring parents to recall details of a behavior that was practiced a minimum of 7 years prior to the data collection. Breastfeeding history was a greater disadvantage to participants who were adopted, as the adoptive parents may not know details of the child's perinatal nutrition. Furthermore, during the data collection appointment, participants and their parents recalled the frequency of consumption of breakfast over the previous year. Accuracy of data was dependent on either the child correctly recalling intake, or the parent having complete knowledge of the diet.

Another limitation was the narrow target population of obese children and adolescents in Salt Lake County. This study population was not representative of obese children and adolescents in the United States, as the sample population consisted of mostly Hispanic and non-Hispanic individuals. Other limitations included the omission of nutrition questions during some data collection visits (requiring a follow up phone call or email), participant refusal of the blood draw and an inability to recruit the goal of 220 volunteers. Transportation to the data collection site during school and working hours was also a barrier to some families, as was transportation during inclement winter weather, thus limiting participation.

The analyses were limited by the lack of socioeconomic status and parent education level reporting. These two factors may play a role in the decision of a parent to breastfeed or not, and may influence overall diet and potentially the A/L ratio. Those mothers who breastfed longer may understand the role of child nutrition in growth, and the A/L ratio of this group might have been affected by an overall better diet throughout life.

Conclusion

Results of this study do not support an association between metabolic dysfunction and the dietary factors of breastfeeding in infancy and frequent breakfast intake in obese children and adolescents. In addition, A/L ratio was not associated with either metabolic dysfunction or the diet habits of interest in the study population. A comparison of increasing breakfast intake and A/L ratio revealed an interesting association when ethnicity was observed, which was not statistically significant. Further investigation into this trend is warranted, as well as continued investigations into the relationship between diet habits, metabolic dysfunction, and A/L ratio in obese youth.

APPENDIX: GOLD MEDAL SCHOOL

PARENT QUESTIONAIRE

30. It is okay for teachers to use food (including candy) as a reward or incentive for students.

a. Strongly disagree
b. Disagree
c. Uncertain
d. Agree
e. Strongly agree

For the following question on child health please fill in each blank with the appropriate number.

31. How concerned are you about each of the following items: (1= Not concerned, 2= Somewhat concerned, 3= Very concerned)

____ Contagious diseases	____ PE classes
____ Childhood smoking	____ Dental health
____ Lice	____ Playground safety
____ Overweight	____ School lunch choices
____ Safety to and from school	____ Other (please specify)
____ Recess time (not enough)	_____

Additional Comments:

Thank you again for taking this survey!

School Health Survey
Parent Questionnaire

Tooele County
Department of Health
and
Utah Department of Health

Dear Parent:

We are trying to learn how schools affect the health of children. We are asking for your help to find out more about this by having you fill out this survey. Please complete the survey for the child who brought it home.

All information will be kept confidential.

Please return this survey to your child's teacher by May 17th. Thank you.

If you have questions, please contact Liz Swan at the Tooele Department of Health at (435) 843-2310.

Who is the main person completing this form?
Circle only one letter.

a. Mother
b. Other Adult Female
c. Father
d. Other Adult Male

Please circle only one answer per question.

Please tell us about your child and your family:

1. Child's grade in school: 1 2 3 4 5 6

2. How many adults over age 18 live in your house?
   a. 1-2
   b. 3-4
   c. 5-6
   d. 7 or more

3. How many children under age 18 live in your house?
   a. 1-2
   b. 3-4
   c. 5-6
   d. 7 or more

4. How do you describe your child? **Optional**
   a. American Indian or Alaska Native
   b. Native Hawaiian or Pacific Islander
   c. Asian
   d. Black or African American (non-Hispanic)
   e. Hispanic
   f. White or Caucasian (non-Hispanic)
   g. Other _____

5. What is the highest level of formal education completed in your family? **Answer only for adults living in the home.**

a. Child's mother (or other adult female)
   a. Less than high school
   b. High school graduate
   c. Some college
   d. College graduate
   e. Graduate degree

b. Child's father (or other adult male)
   a. Less than high school
   b. High school graduate
   c. Some college
   d. College graduate
   e. Graduate degree

26. What students eat during the school day affects how ready they are to learn.
   a. Strongly disagree
   b. Disagree
   c. Uncertain
   d. Agree
   e. Strongly agree

27. Students should be able to buy soft drinks and candy at school.
   a. Strongly disagree
   b. Disagree
   c. Uncertain
   d. Agree
   e. Strongly agree

28. Selling high-fat, high-sugar foods, such as candy and cookies, as part of school fundraising is okay because it helps raise money for school programs.
   a. Strongly disagree
   b. Disagree
   c. Uncertain
   d. Agree
   e. Strongly agree

29. It is important for schools to have a written school food policy about things like food in the classroom or snack and drink choices in vending machines.
   a. Strongly disagree
   b. Disagree
   c. Uncertain
   d. Agree
   e. Strongly agree

11. How many hours does your child usually spend on a typical **WEEKDAY** playing video or computer games?
- a. Less than 1 hour
- b. 1 hour
- c. 2 hours
- d. 3 hours
- e. 4 hours
- f. 5 hours or more

12. How many hours does your child usually spend on a typical **WEEKEND DAY** watching television or videos? Include DVD and video movies. Do not count video games.
- a. Less than 1 hour
- b. 1 hour
- c. 2 hours
- d. 3 hours
- e. 4 hours
- f. 5 hours or more

13. How many hours does your child usually spend on a typical **WEEKEND DAY** playing video or computer games?
- a. Less than 1 hour
- b. 1 hour
- c. 2 hours
- d. 3 hours
- e. 4 hours
- f. 5 hours or more

14. How safe is it for your child to walk to school?
- a. Very unsafe
- b. Somewhat unsafe
- c. Not sure
- d. Somewhat safe
- e. Very safe

15. How safe is it for your child to play outdoors with other children in your neighborhood without adult supervision?
- a. Very unsafe
- b. Somewhat unsafe
- c. Not sure
- d. Somewhat safe
- e. Very safe

16. During the past week, how often has an adult in your family encouraged your child to do physical activities or play sports?
- a. Not in the past week
- b. Once
- c. A few times
- d. Often

17. During the past week, how often has an adult in your family done a physical activity or played sports with your child?
- a. Not in the past week
- b. Once
- c. A few times
- d. Often

Eating Habits

18. How many times in an average week does your child eat breakfast?
- a. 0
- b. 1
- c. 2
- d. 3
- e. 4
- f. 5
- g. 6
- h. 7

19. How many times in an average week does your family eat outside the home?
- a. 0 times
- b. 1-2 times
- c. 3-4 times
- d. 5-6 times
- e. Every day
- f. More than once a day

20. How many soft drinks does your child drink on a typical day? (1 drink = a tall glass or 12 oz can) Do not include diet soft drinks.
- a. 0
- b. 1
- c. 2
- d. 3 or more

For questions (21-30), please evaluate the following statements:

21. I feel that I am able to provide nutritious meals for my child and family.
    a. Strongly disagree
    b. Disagree
    c. Uncertain
    d. Agree
    e. Strongly agree

22. It is important to address eating habits during childhood.
    a. Strongly disagree
    b. Disagree
    c. Uncertain
    d. Agree
    e. Strongly agree

23. What parents eat affects what their children eat.
    a. Strongly disagree
    b. Disagree
    c. Uncertain
    d. Agree
    e. Strongly agree

24. The nutritional health of students should be important to schools.
    a. Strongly disagree
    b. Disagree
    c. Uncertain
    d. Agree
    e. Strongly agree

25. Only healthful foods should be available at school.
    a. Strongly disagree
    b. Disagree
    c. Uncertain
    d. Agree
    e. Strongly agree

6. Please describe your child's weight.
    a. Extremely underweight
    b. Somewhat underweight
    c. About right
    d. Somewhat overweight
    e. Extremely overweight

Physical Activity

7. How many days in an average week does your child walk or bike to school?
    a. 0 days
    b. 1 day
    c. 2 days
    d. 3 days
    e. 4 days
    f. 5 days

8. At least once a week, does your child engage in regular activity like brisk walking, jogging, and bicycling, long enough to work up a sweat?
    a. Yes
    b. No

9. If yes to number 8, how many days per week?
    a. 1 day
    b. 2 days
    c. 3 days
    d. 4 days
    e. 5 days
    f. 6 days
    g. 7 days

10. How many hours does your child usually spend on a typical **WEEKDAY** watching television or videos? Include DVD and video movies. Do not count video games.
    a. Less than 1 hour
    b. 1 hour
    c. 2 hours
    d. 3 hours
    e. 4 hours
    f. 5 hours or more

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